Experimental and Clinical Studies on the Effect of Essential Fatty Acid Deficiency on Adrenocortical Capacity

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by

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PART I　EXPERIMENTAL STUDY

1. INTRODUCTION

Since the classical discovery of Burr and Burr that certain polyenoic fatty acids, such as linoleic acid, linolenic acid and arachidonic acid, which cannot be synthesized in the animal body, are essential for growth and survival, it has been more generally recognized that fat may serve to maintain cell function not only as a source of energy but also as one of the important elements of cell structure. Among these polyenoic acids, linolenic acid improved incompletely the fat deficiency syndromes. Accordingly, nowadays, this fatty acid has been excluded from the category of essential fatty acids (here after abbreviated as EFA), and only the fatty acids derived from linoleic acid have been regarded as EFA. It is known that especially arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid) improves the fat deficiency syndromes three times more effectively than linoleic acid, and ultimately plays the peculiar role of an EFA in the body. So, up to the present time, the numerous biochemical studies with respect to EFA have been studied by many investigators, but the functional roles and metabolic process of EFA in the body have not yet been sufficiently clarified. For example, it may be said that the relationship between EFA and steroid hormone metabolism is hardly solved.

Until recently, in our laboratory, the specific physiological actions of EFA have been studied from the surgical standpoint, and owing to the high concentrations of EFA as well as of cholesterol in the adrenals, it was surmised that EFA was related to the cholesterol metabolism in the adrenals and played an important role on the biosynthesis of adrenocortical hormone, a precursor of which was adrenal cholesterol. Our colleague MATSUDA's experiments on the light microscopic and histochemical observations of the adrenal cortex, and on the change of liver glycogen content during fasting, indicated that the deficiency in EFA was an important factor from which the adrenocortical capacity was reduced. ISHIMARU's work on the electron microscopic study of the adrenal cortex, indicated that the extreme exhaustive changes in the adrenocortical fasciculata cell of EFA deficient animals were found under various stresses. KUMANO reported that only when the adrenal

cholesterol was sufficiently esterified with EFA, could the normal adrenocortical capacity of the organism be maintained. And TAMAKI, measuring the levels of plasma fluorometric corticosteroids and the excretion of urinary formaldehydogenic corticosteroids of rats under various stresses, showed distinctly that the adrenocortical function of the EFA deficient rats was much poorer than that of the EFA sufficient rats.

Then, in earlier numerous experimental studies, the adrenal weight, histologic investigations, plasma and urinary corticosteroid levels at resting state have been used mainly to estimate the adrenocortical function. Therefore, it has not been necessarily easy to reach the precise conclusion. Thus in the present study, the changes of serum and adrenal corticosterone levels in response to ACTH administration were investigated, and based on the results an attempt was made to demonstrate directly the adrenocortical capacity. By this method the effect of EFA on the biosynthesis of adrenocortical hormone, especially glucocorticoid, was studied.

At the same time, the effect of vitamin B_6 on EFA metabolism was investigated from the view-point of the adrenocortical capacity.

2. EXPERIMENTAL ANIMALS

Male albino rats of Wistar Strain supplied by the Animal Center of Kyoto University were used for the experiment. The weaned rats were fed on a standard diet (rat chow) until their boby weight reached about 50～60 g, then they were divided into the following three groups:

1) Fat diet group
2) Fat deficient diet group
3) Vitamin B_6 deficient fat diet group

The first group was fed on a fat diet for 12 weeks, the second, on a fat deficient diet for 12 weeks and the third, on a fat diet for 6 weeks and then on a vitamin B_6 deficient fat diet for 6 weeks. These rats were fed *ad libitum* the experimental diets and kept at a constant room temperature of 20±3°C, then were used for the experiment.

The weight composition of each diet is shown in Table 1. That is, according to the gasliquid chromatographic analysis in our laboratory, the purified sesame oil used as the source

Table 1 Composition of the Diets and Fatty Acid Composition in the Sesame Oil Determined by GLC

	Fat diet	Fat deficient diet	Vit. B_6 deficient fat diet	Sesame oil	
Starch	60%	80%	60%	Fatty acid	%
Casein	20	20	20	16 : 0	9.5
				16 : 1	0.7
Sesame oil	20	0	20	18 : 0	5.1
Salt mixture	3	3	3	18 : 1	34.6
Vitamin mixture	0.5	0.5	0.5 (Vit. B_6 free)	18 : 2	48.9
				18 : 3	0.7
Choline chloride	0.5	0.5	0.5	20 : 0	0.5
				20 : 4	0

of EFA contained a linoleic acid of 48.9%, but no arachidonic acid. The EFA contents in the casein and starch were practically negligible, therefore, the fat deficient diet used in this experiment may be regarded as the EFA free diet.

3. EXPERIMENTAL METHODS

It has been generally known that serum and adrenal corticosterone levels of rats tend to vary with such factors as season, time of day, age, and feeding conditions, etc.

Since the corticosteroidogenic response is very sensitive to changes of any kind, much care was taken not to expose the rats to unnecessary environmental disturbances, and all groups were maintained at approximately the same conditions during the course of feeding. Prior to the experiment these rats were placed in individual cages for several days and then sacrificed after fasting for 12 hours. The rats were anesthetized by an intraperitoneal injection of nembutal.

After laparotomy, blood was taken from the abdominal aorta and bilateral adrenalectomy was immediately performed, then the serum was separated from the collected blood.

As the difference between left and right adrenal corticosterone content is negligible, one adrenal was used for measurement of corticosterone, the other, for determination of fatty acids. The separated serum and excised adrenals were immediately preserved in the refrigerator and then were used for experiment.

i) Method for Determination of Adrenal Corticosterone

A modification of SILBER's and ZENKER's fluorometric method, which was improved by IMURA, was used for the determination of adrenal corticosterone.

As the fluorescence of corticosterone is developed at room temperature in this method, the influence of temperature must be considered (Table 2).

Table 2 Effect of Temperature on the Development of Fluorescence

Room Temperature	Time Developed Maximum Fluorescence	Time Continued Maximum Fluorescence
10°C	120 min.	120 min.
15	90	60
20	60	30
25	30	20
30	20	10

The withdrawal of corticosterone ranged from 90% to 105%, with 95% as the average.

Reagents:

1) Chloroform (C. P. grade)
2) Petroleum Ether (C. P. grade)
3) 0.1 N NaOH
4) 50% Ethyl Alcohol (Wako Co. Ltd.)
5) Sulfuric Acid (C. P. grade Mitsubishikasei Co. Ltd.)
6) Fluorescence Reagent: Add 2.4 volumes of sulfuric acid to one volume of 50% Ethyl Alcohol.
7) Standard Reagent: Dissolve 1 μg of corticosterone in 1 ml of distilled water.

Procedure:

An adrenal gland of the rat was crushed with 1 ml of 50% ethanol in a glass homo-

genizer, then diluted to 12 ml with distilled water. A 4 ml of the adrenal solution was washed with 3 volumes of petroleum ether by shaking for 30 seconds. After centrifugation at 2500 r. p. m. for 5 minutes, the bulk of the aquous phase was carefully removed by aspiration and discarded.

Then extraction from 3 ml of adrenal solution was immediately carried out with 15 ml of chloroform by shaking for 30 seconds and then centrifugation was done for 5 minutes at 2500 r. p. m..

The adrenal solution layer was removed and discarded. A 1.5 ml of 1/10 N NaOH was added to the chloroform extract. The chloroform extract was quickly shaken for 15 seconds and centrifuged for 5 minutes at 2500 r. p. m. and then, the alkaline wash was discarded. A 4.5 ml of the fluorescence reagent was added to 10 ml of the chloroform extract. After shaking for 30 seconds and centrifuging for 5 minutes at 2500 r. p. m. the chloroform layer was discarded.

According to the room temperature, after from 20 minutes to 2 hours, the fluorescence of the sample was measured by a photoelectric colorimeter (SHIMAZU QB-50) at an exciting wave length of 450 mμ (Filter: K-7) and at an emitted wave length of 520 mμ (Filter: YA-3).

As a standard reagent, 1 μg of corticosterone dissolved in 1 ml of distilled water was used. As a blank, distilled water was used (Fig. 1).

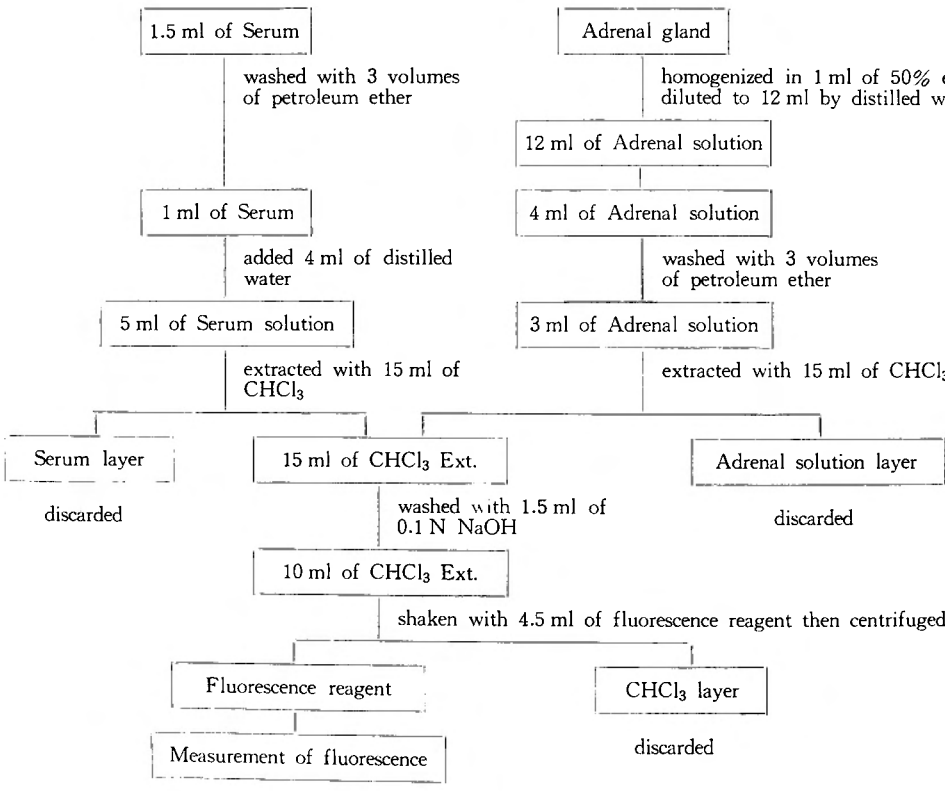

Fig. 1 Fluorometric Methods for Determination of Serum and Adrenal Corticosterone

ii) Method for Determination of Serum Corticosterone

A 1.5 ml of serum was washed with 3 volumes of petroleum ether, then 1 ml of the serum was diluted to 5 ml with distilled water, and the same procedure as described above was carried out throughout the process (Fig. 1).

4. RESULTS

i) Effect of a Dietary Fat Deficiency on the Adrenocortical Activity of Rats

Rats in the fat diet group grew at a normal rate during the course of feeding, while rats in the fat deficient diet group were markedly retarded in their growth. After 12 weeks of feeding on respective diets, the difference of body weight between rats in these two groups was remarkable. That is, the former was 282 ± 12 g, while the latter was 166 ± 6 g. Furthermore, numerous premature deaths in the fat deficient diet group occurred during the course of feeding.

Adrenal weight, serum and adrenal corticosterone contents were apparently greater in the fat diet group than in the fat deficient diet group (Table 3). With the exclusion of EFA from the diet, it was demonstrated that the adrenal dienoic acid and tetraenoic acid contents were always decreased, while only trienoic acid was increased, and such a pattern of adrenal polyenoic acid composition was characteristic of EFA deficiency (Table 4). Then, about 80% of adrenal tetraenoic acid contents in both groups was esterified with cholesterol. The cholesterol ester tetraenoic acid contents were greater in the fat diet group than in the fat deficient diet group and about 30% of the fatty acids esterified with cholesterol was tetraenoic acid in the former, while only 10 % of that was tetraenoic acid in the latter. And the adrenal total cholesterol contents were somewhat greater in the fat deficient diet group than in the fat diet group.

Furthermore, when the adrenal polyenoic acid composition, which was analyzed by the gas-liquid chromatography, was compared with the results, which were determined by the alkaline isomerization method, it was clarified that the adrenal dienoic acid represented mainly linoleic acid (C 18 : 2), tetraenoic acid represented mainly arachidonic acid (C 20 : 4) and 7, 10, 13, 16-docosatetraenoic acid (C 22 : 4), and the trienoic acid represented not only linolenic acid (C 18 : 3) but also 5, 8, 11-eicosatrienoic acid (C 20 : 3) and 7, 10, 13-docosatrienoic acid (C 22 : 3) (*MURAOKA*).

The parallel relationship between the adrenocortical activity and the adrenal tetraenoic acid contents esterified with cholesterol was found even at resting state.

ii) Effect of a Single Injection of ACTH-Z on Serum and Adrenal Corticosterone Levels of Rats

Recently it has become known that there is partial or compensated adrenal insufficiency, in spite of the normal levels of plasma or urinary corticoids at rest, and also that there is sufficient adrenal response to ACTH stimulation, in spite of the low levels of plasma and urinary corticoids at rest. Therefore, the precise evaluation of adrenocortical capacity should be estimated not only from the plasma and urinary corticoid excretion levels at resting state, but also from the increment of corticoids in response to ACTH stimulation.

Then, a single injection of ACTH-Z 3 I. U. in the back muscle was given to the rats in the fat diet group and fat deficient diet group. Its results were as follows: the

Table 3 Effect of Dietary Fat Deficiency on Adrenocortical Activity of Rats

	No.	Body Weight (mg)	Adrenal Weight (mg)	Adrenal Corticosterone (μg/adrenal)	Adrenal Corticosterone (μg/100mg adr.)	Serum Corticosterone (μg/100 ml)
Fat diet group	1	350	30	1.24	4.13	39
	2	250	23	1.08	4.69	
	3	320	22	0.82	3.72	
	4	260	24	0.68	2.83	42
	5	300	28	0.64	2.28	
	6	310	30	0.92	3.06	
	7	315	20	0.68	3.40	34
	8	365	24	0.84	3.50	
	9	270	18	0.72	4.00	40
	10	230	18	0.72	4.00	
	11	220	24	0.82	3.41	42
	12	280	22	0.88	4.00	
	13	210	21	0.64	3.04	
	14	270	26	0.76	2.92	
	Mean±S. E.	282±12	23.5±1.0	0.81±0.05	3.49±0.16	39.4±1.9
Fat deficient diet group	1	130	15	0.28	1.86	33
	2	150	14	0.44	3.11	
	3	150	14	0.36	2.57	
	4	180	17	0.50	2.94	25
	5	180	18	0.52	2.88	
	6	170	18	0.52	2.88	
	7	130	20	0.50	2.50	25
	8	185	17	0.54	3.17	
	9	160	18	0.52	2.88	
	10	170	33	0.56	1.69	30
	11	210	18	0.54	3.00	
	12	150	18	0.48	2.66	
	13	200	14	0.24	1.71	39
	14	160	16	0.34	2.12	
	15	170	20	0.42	2.10	
	Mena±S .E.	166±6	18.0±1.2	0.45±0.09	2.54±0.21	30.4±2.6

Table 4 Effects of Dietary EFA and Vit. B_6 on Adrenal Polyenoic Acids and Cholesterol Contents of Rats (*MURAOKA*)

	No. of Rats	Adrenal Polyenoic Acids (mg/100 mg adr.)										Adrenal Cholesterol (mg/100mg adr.)	
		Total Polyenoic Acids					Ch-ester Polyenoic Acids						
		Di	Tr	Tt	Pt	Hx	Di	Tr	Tt	Pt	Hx	Total	Esterified
Fat diet group	5	1.10	0.17	1.55	0.14	0.03	0.13	0.12	1.07	0.11	0.03	5.67	4.96
Fat deficient diet group	5	0.19	1.51	0.86	0.17	0.10	0.12	0.96	0.70	0.15	0.09	8.22	7.70
Vit. B_6 deficient fat diet group	5	0.45	0.16	1.79	0.18	0.04	0.13	0.11	1.51	0.15	0.03	5.72	5.07

serum and adrenal corticosterone levels in both groups reached their peak 1 or 2 hours after ACTH injection.

However, there was a great difference between the adrenocortical capacity in both groups. That is, the adrenocortical capacity of the organism was severely reduced with the deficiency in EFA (Table 5, Fig. 2).

At this time, the adrenal cholesterol ester arachidonic acid content in the fat diet group was once decreased by ACTH injection, and then gradually recovered to the former

Table 5 Effect of Single ACTH-Z Injection on Serum and Adrenal Corticosterone of Rats

	After ACTH Inj.	No. of Rats	Body Weight (g)	Adrenal Weight (mg)	Serum Corticosterone (μg/100 ml)	Adrenal Corticosterone (μg/adrenal)	Adrenal Corticosterone (μg/100mg adr.)
Fat diet group	0 hrs.	6	310± 8*	22.0±0.9	40	0.84±0.09	3.52±0.39
	1	5	280±20	22.2±0.8	100	1.20±0.03	5.04±0.27
	2	5	303± 7	23.7±0.3	92	1.30±0.07	5.50±0.24
	4	5	306±18	25.0±1.1	56	0.82±0.10	3.52±0.48
	6	5	325± 6	24.5±0.6	44	0.60±0.05	2.48±0.39
	12	5	317± 7	22.0±0.8	25	0.42±0.05	1.73±0.25
Fat deficient diet group	0	6	158±12	17.5±0.5	25	0.51±0.03	2.98±0.46
	1	5	155± 8	15.3±0.7	57	0.67±0.06	4.43±0.21
	2	5	152± 7	15.0±0.1	54	0.75±0.09	5.06±0.48
	4	5	167± 4	14.7±0.4	34	0.34±0.10	2.30±0.19
	6	5	145±14	15.2±0.8	24	0.29±0.67	1.98±0.24
	12	5	155± 5	15.3±0.7	22	0.23±0.04	1.52±0.35

*Standard error of the mean.

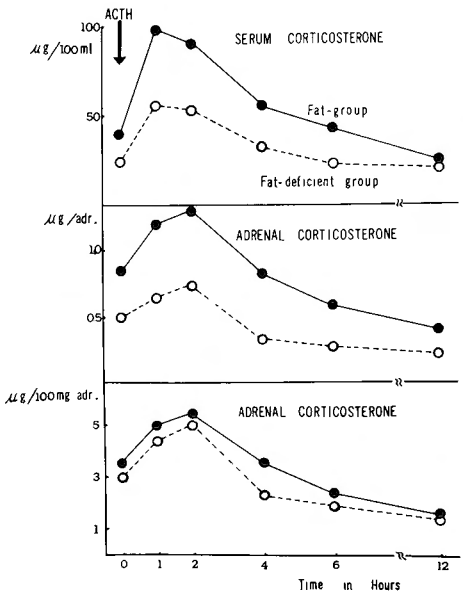

Fig. 2

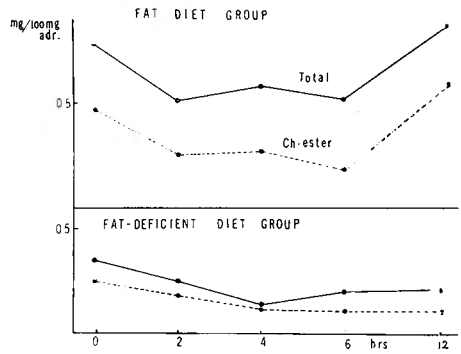

Fig. 3 Changes in Adrenal Total and Cholesterol Ester Arachidonic Acid Contents after Injection of ACTH-Z 3 I. U. (*MURAOKA*)

level before ACTH injection. On the other hand, that in the fat deficient diet group was rapidly decreased by ACTH injection, which then developed to the exhaustive state (Fig. 3).

Two hours after ACTH injection, when the adrenal corticosterone level had reached its peak, the arachidonic acid content in the adrenal cholesterol ester fatty acids in both groups was significantly decreased in percentage more than the other fatty acids.

From the results mentioned above, it was demonstrated that the degree of adrenocortical capacity of the organism is not ultimately decided from the adrenal total and esterified cholesterol, but from the adrenal arachidonic acid content esterified with cholesterol.

iii) Effect of Cold Stress on the Serum and Adrenal Corticosterone Levels of Rats

It is known that the acute exposure to cold actually acts to produce an increased amount of corticotrophin, which stimulates the adrenal cortex as a typical stressor. And the acting mechanism of cold stress to adrenal cortex is thought to be essentially identical to that of ACTH stimulation, as far as the pituitary functions are normally maintained.

The rats in the fat diet group and fat deficient diet group were immersed in ice water for a moment and then kept in a refrigerator at a temperature of $-10°C$ for 30 minutes. The changes of serum and adrenal corticosterone levels in both groups were observed. However, the serum and adrenal corticosterone levels in both groups reached their peak 1 hour after cold stress and then gradually decreased (Table 6). These changes of corticosterone levels were similar to the pattern observed in the rats treated with a single ACTH injection.

In this experiment, also, it was found that the adrenocortical capacity was better maintained in the fat diet group than in the fat deficient diet group.

iv) Effect of Vitamin B_6 Deficiency on Serum and Adrenal Corticosterone Levels of Rats

An intimate relationship between vitamin B_6 and the metabolism of EFA in the body has been demonstrated by numerous investigators, and it has recently been reported that this vitamin is concerned with the conversion of linoleate to arachidonate. Therefore, it

Table 6 Effect of Cold Stress on Serum and Adrenal Corticosterone of Rats

	After Cold	No. of Rats	Body Weight (g)	Adrenal Weight (mg)	Serum Corticosterone (μg/100ml)	Adrenal Corticosterone (μg/adrenal)	Adrenal Corticosterone (μg/100mg adr.)
Fat diet group	0 hrs.	6	310± 8*	22.0±0.9	34	0.84±0.09	3.52±0.39
	1	5	300± 2	23.5±0.5	105	1.25±0.05	7.13±0.15
	2	5	271±13	23.0±1.0	66	1.15±0.07	5.0 ±0.23
	4	5	260±10	24.0±0.5	52	0.83±0.10	3.36±0.27
	6	5	323±15	24.3±0.3	35	0.77±0.08	3.21±0.46
Fat deficient diet group	0	6	158±12	17.5±0.5	25	0.51±0.03	2.98±0.46
	1	5	160± 2	13.5±0.3	45	0.59±0.09	4.53±0.33
	2	5	147±15	14.0±0.7	40	0.42±0.04	3.24±0.25
	4	5	133± 7	12.9±0.9	28	0.33±0.07	2.56±0.46
	6	5	143± 8	14.5±0.2	22	0.25±0.04	1.90±0.24

*Standard error of the mean.

is presumed that the deficiency in vitamin B_6 has a secondary influence on the biosynthesis of adrenocortical hormone through the metabolic disturbances in EFA.

Then, the vitamin B_6 deficient fat diet group was experimentally prepared, and the effect of a single injection of ACTH-Z to this group was discussed, as compared with that to the vitamin B_6 supplemented fat diet group.

However, as vitamin B_6 is produced also from the intestinal flora the results from this experiment are not regarded as those under the absolute deficient state of vitamin B_6, but under the relative deficient state of vitamin B_6. The rats fed on the vitamin B_6 deficient fat diet were markedly retarded in their growth, but their adrenal weight and serum corticosterone levels were almost equal to those of the rats fed on the vitamin B_6 supplemented fat diet. However, the adrenal corticosterone levels were distinctly lower in the former group than in the latter group even at rest. Therefore, partial adrenocortical insufficiency in the vitamin B_6 deficient diet group was surmised (Table 7).

Table 7 Effect of Vit. B_6 Deficiency on Serum and Adrenal Corticosterone of Rats

	After ACTH Inj.	No. of Rats	Body Weight (g)	Adrenal Weight (mg)	Serum Corticosterone (μg/100ml)	Adrenal Corticosterone (μg/adrenal)	Adrenal Corticosterone (μg/100mg adr.)
Vit. B_6 supplemented fat diet group	0 hrs.	5	279± 9*	18.5±1.1	39	0.80±0.09	4.33±0.20
	2	5	297± 3	22.0±0.9	88	1.08±0.07	6.05±0.12
	4	5	280± 5	16.5±1.2	61	0.76±0.14	3.94±0.41
	6	5	248± 2	19.5±0.5	42	0.54±0.06	3.82±0.58
Vit. B_6 deficient fat diet group	0	5	157± 5	18.5±1.5	36	0.60±0.03	3.32±0.18
	2	5	154± 4	18.0±1.6	83	0.85±0.03	4.74±0.19
	4	5	170±14	20.0±0.2	48	0.63±0.12	3.18±0.29
	6	5	111±12	18.0±0.7	34	0.48±0.05	2.68±0.45

*Standard error of the mean.

Then, daily, four successive injections of ACTH-Z 3 I. U. were given to the rats fed on the vitamin B_6 supplemented fat diet, vitamin B_6 supplemented fat deficient diet, and vitamin B_6 deficient fat diet respectively. And the changes of the serum and adrenal corticosterone levels 2 hours after ACTH injection were observed. In the vitamin B_6 supplemented fat diet group, the serum and adrenal corticosterone response to successive injections of ACTH-Z was sufficient even after the fourth injection of ACTH-Z as well as at the first. While in the vitamin B_6 supplemented fat deficient diet group, the serum and adrenal corticosterone response to ACTH injection was gradually decreased as ACTH injections were repeated.

And at first, in the vitamin B_6 deficient fat diet group, the serum corticosterone response to ACTH injection was almost equal to that of the vitamin B_6 supplemented fat diet group. However, the response was decreased as ACTH injections were repeated and then approached that of the vitamin B_6 supplemented fat deficient diet group. Furthermore, in the vitamin B_6 deficient fat diet group, the change of the adrenal corticosterone content, induced by the successive injections of ACTH, was between that of the vitamin B_6 supplemented fat diet group and that of the vitamin B_6 supplemented fat deficient diet group (Fig. 4), (Table 8).

At this time, in the vitamin B_6 supplemented fat diet group the total arachidonic acid and cholesterol ester arachidonic acid contents in the adrenals were sufficient even after the fourth injection of ACTH as well as at first. While, in the vitamin B_6 supplemented fat deficient diet group, those in the adrenals were extremely diminished as the ACTH injections were repeated. And in the vitamin B_6 deficient fat diet group the adrenal arachidonic acid in total as well as in esterified form was sufficient as in the vitamin B_6 supplemented fat diet group at the first injection of ACTH, but not at fourth injection of ACTH. Nevertheless the total was sufficient, the esterified was between that of the vitamin B_6 supplemented fat diet group and that of the vitamin B_6 supplemented fat deficient diet group, decreasing extremely its contents in the adrenals (Fig. 5). These changes were similar to the pattern observed in the changes of serum and adrenal corticosterone levels by the four successive injections of ACTH.

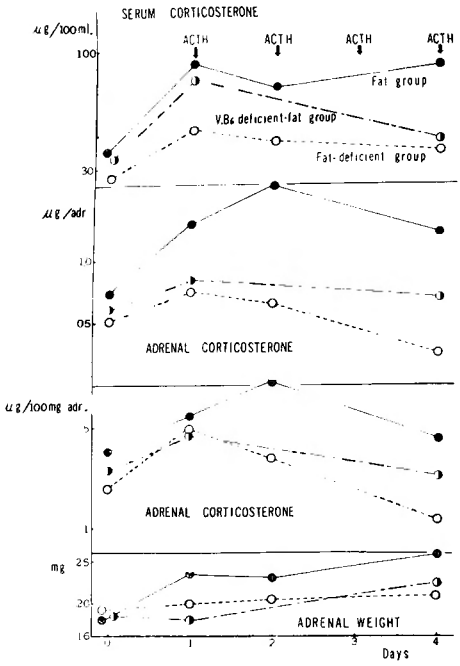

Fig. 4

Therefore, it was surmised that, in the deficient state of vitamin B_6, the disturbance

Table 8 Adrenocortical Activity Following the Daily Four Successive Injections of ACTH-Z

		No. of Rats	Body Wieght (g)	Adrenal Weight (mg)	Serum Corticosterone (μg/100ml)	Adrenal Corticosterone (μg/adrenal)	Adrenal Corticosterone (μg/100mg adr.)
Vit. B_6 supplemented fat	Control	5	243± 8*	18.0±0.1	40	0.72±0.08	4.02±0.12
	ACTH-Z 3 I.U. 1 day	5	303± 7	23.7±0.3	92	1.30±0.07	5.50±0.24
	ACTH-Z 3 I.U. 2 days	5	212±12	23.0±1.2	79	1.60±0.03	6.94±0.16
	ACTH-Z 3 I.U. 3 days	—	—	—	—	—	—
	ACTH-Z 3 I.U. 4 days	5	209± 6	26.0±1.8	91	1.24±0.06	4.66±0.24
Vit. B_6 supplemented fat deficient	Control	5	185±15	19.3±0.7	25	0.52±0.08	2.64±0.15
	ACTH-Z 3 I.U. 1 day	5	152± 7	20.0±1.0	54	0.75±0.09	5.06±0.48
	ACTH-Z 3 I.U. 2 days	5	151±13	20.5±0.5	46	0.68±0.02	3.76±0.34
	ACTH-Z 3 I.U. 3 days	—	—	—	—	—	—
	ACTH-Z 3 I.U. 4 days	5	155±15	21.0±0.9	40	0.28±0.05	1.42±0.22
Vit. B_6 deficient fat	Control	5	157± 5	18.5±1.5	36	0.60±0.03	3.32±0.18
	ACTH-Z 3 I.U. 1 day	5	154± 4	18.0±1.6	83	0.85±0.03	4.74±0.19
	ACTH-Z 3 I.U. 2 days	—	—	—	—	—	—
	ACTH-Z 3 I.U. 3 days	—	—	—	—	—	—
	ACTH-Z 3 I.U. 4 days	5	160± 7	22.5±0.6	47	0.71±0.06	3.12±0.44

*Standard error of the mean.

in the process of arachidonic acid esterification with cholesterol caused secondarily the suppression of adrenocortical capacity.

5. DISCUSSION

Since ZAFFARONI has proved that the adrenocortical hormone is synthesized from the acetate and cholesterol, the numerous studies with regard to the biosynthesis of this hormone have been reported. It is generally known that about 10% of cholesterol is contained in the adrenal cortex and its major part is esterified. And it is rapidly decreased under various kinds of stresses or by ACTH administration and one fourth of it is used in the biosynthesis of adrenocortical hormone.

While with respect to EFA in the adrenals it was suggested by SINCLAIR that EFA may be concerned with the synthesis of steroid hormone from cholesterol since it is abundantly contained in the adrenal cortex, testes and ovaries. HAYASHIDA & PORTMAN have reported that the secretion of adrenocortical hormone under the influence of ACTH in vitro was less with adrenals from rats deficient in polyunsaturated fatty acids than with those from rats, that had received corn oil.

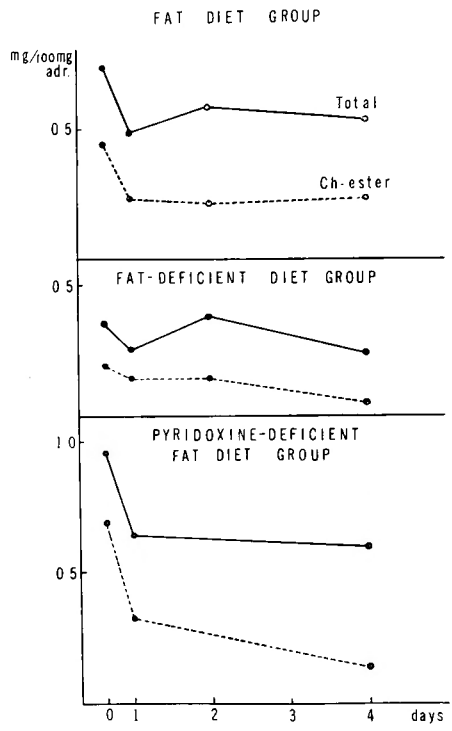

Fig. 5 Changes in Adrenal Arachidonic Acid Contents During the Daily Four Successive Injections of ACTH-Z (*MURAOKA*)

On the other hand, after several years, in our laboratory also, becoming aware of the importance of EFA in the adrenals, its physiological significances in vivo have been investigated by means of histological and biochemical analysis. The results from these experiments have clarified that the adrenal cholesterol was activated only when it was esterified with EFA, especially with arachidonic acid, then adrenocortical hormone was smoothly synthesized from the adrenal cholesterol.

And from the same standpoint, our colleagues YOSHINAGA & MARUYAMA surmised that the deficiency in EFA should cause necessarily the change of bile acids, which are normal end products of cholesterol, in quantity and quality, and such changes might become initiating factors of cholesterol gallstone formation. And they have experimentally succeeded to verify the justice of such concepts.

Furthermore, a number of investigators have suggested that there is an intimate relationship between vitamin B_6 and EFA metabolism. WITTEN & HOLMAN reported that the conversion of linoleate to arachidonate was impaired in the essential fatty acid-pyridoxine deficient rat. DAM et al. have made similar observations in chicks. GOSWAMI and SADHU also reported that in pyridoxine deficient rats there was a lower serum tetraenoic acid level compared with normal animals.

Moreover, TAMAKI in our laboratory has proved that the urinary formaldehydogenic corticoid level in rats was decreased in the state of vitamin B_6 deficiency, and ISHIHARA has reported the lower plasma corticoid concentrations in rats with this vitamin deficiency.

In the present study, the investigation was carried out with rats fed on the relative vitamin B_6 deficient and linoleate supplemented diet. And the results from this animal experiment demonstrated very clearly that the adrenocortical capacity in the vitamin B_6 deficient rats was certainly lower compared with the control, and at this time, the deficiency in this vitamin did not impare the conversion of linoleate to arachidonate, but through the disturbance in the process of cholesterol esterification with arachidonic acid, the adrenocortical capacity was secondarily reduced.

6. CONCLUSION

The effects of the EFA and vitamin B_6 deficiency on the biosynthesis of glucocorticoids in the adrenals of rats were investigated:

1) In the EFA deficient state, the corticosterone in the serum as well as in the adrenals of the organism was apparently reduced in its concentrations even at rest, and at the same time, the amount of cholesterol ester arachidonic acid in the adrenals was decreased, in spite of the sufficient total and esterified cholesterol contents.

2) The adrenocortical capacity, which was estimated from the increment of serum and adrenal corticosterone response to a single injection of ACTH and cold stress, was greatly reduced in the EFA deficient organism.

Generally the adrenal total arachidonic acid and cholesterol ester arachidonic acid contents of organism were decreased, contrary to the change of adrenal corticosterone, under various kinds of stresses or by the administration of ACTH. And at this time, those in the EFA deficient organism were developed to the exhaustive state, while those in the EFA sufficient organism were once decreased and then rapidly recovered to the former levels, and never developed to the exhaustive state.

3) At first, with the daily, four successive injections of ACTH, the serum and adrenal corticosterone levels in the vitamin B_6 deficient and EFA supplemented diet group were almost equal to those in the vitamin B_6 and EFA supplemented diet group, and then gradually decreased. At the fourth injection the response to ACTH approximated that of the vitamin B_6 supplemented and EFA deficient diet group.

Then, at the first injection of ACTH, in the vitamin B_6 deficient and EFA supplemented diet group, the total and cholesterol ester arachidonic acid contents in the adrenals were as sufficient as those in the vitamin B_6 and EFA supplemented diet group. At the fourth injection of ACTH, however, the amount of the former was sufficient, but the amount of the latter was greatly diminished in the adrenals.

4) From the results described above, it was concluded that the total and esterified cholesterol contents in the adrenals did not represent the adrenocortical capacity faithfully, but that the adrenocortical capacity was decided by an amount of arachidonic acid with which adrenal cholesterol esterifies.

PART II CLINICAL STUDY

1. INTRODUCTION

Our colleague JINDO had already reported that an EFA deficient state was found in patients with malignant tumors. Accordingly, it was surmised that the adrenocortical capacity of such patients in EFA deficient state should be necessarily reduced.

Then in the present study, the relationship between serum EFA concentration and the excretions of urinary 17-hydroxycorticoids (abbreviated as 17-OHCS) in surgical patients was investigated. And it was discussed whether the adrenocortical capacity may be necessarily reduced or not by the EFA deficient state in human beings as well as in rats.

2. EXPERIMENTAL METHODS

Plasma concentration and urinary excretions of 17-OHCS are sensitively influenced by the duration and severity of the surgical procedure and anesthesia. Accordingly, to avoid these complicated influences, the ACTH test was performed immediately on the patients admitted to the hospital and at rest in the early morning. For this test, an intramuscular single injection of 20 I. U. of zinc-corticotropin (ACTH-Z) was used.

The serum polyenoic acid concentration was determined before ACTH injection.

i) Method for Determination of Urinary Total 17-Hydroxycorticoids

A modification of Reddy, JENKINS & THORN's method was used for determination of urinary total 17-OHCS excretion values (Fig. 6).

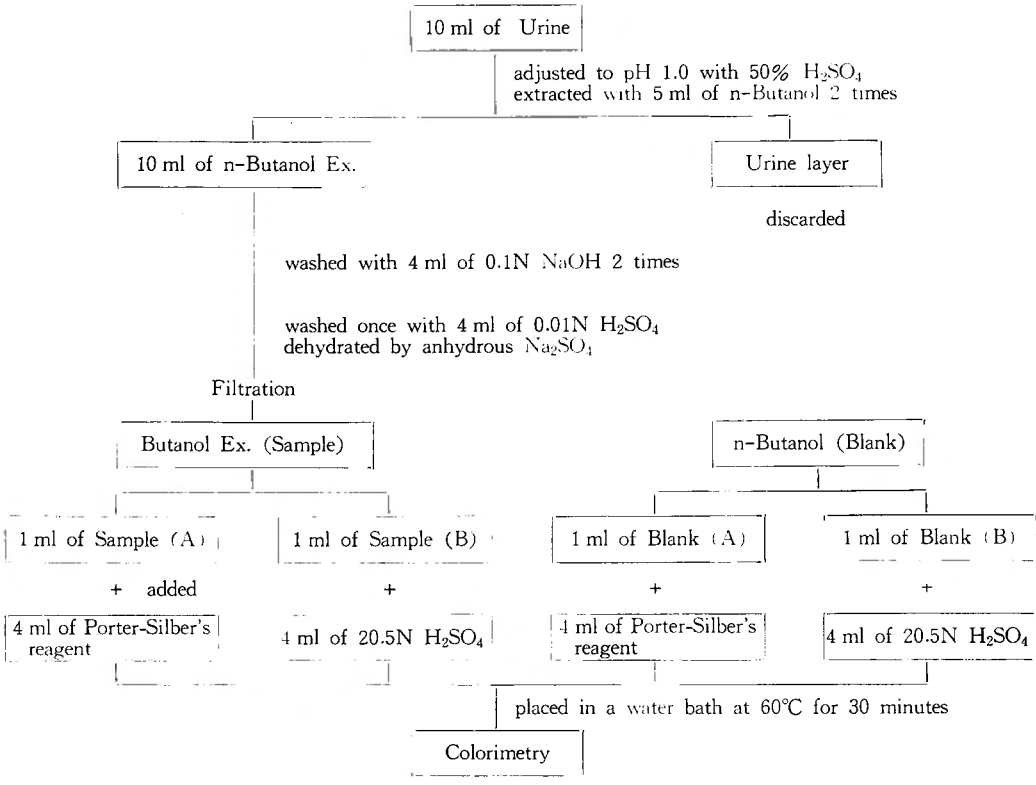

Fig. 6 Method for Determination of Urinary Total 17-OHCS

Reagents :

1) n-Butanol : purificated by TORII's method.
2) Chloroform (C. P. grade).
3) 20.5 N Sulfuric Acid : 560 ml of concentrated H_2SO_4 is diluted to 1 liter with distilled water.
4) Phenylhydrazine-Sulfuric Acid Reagent (Porter-Silber's Reagent) : 65 mg phenylhydrazine hydrochloride is dissolved in 100 ml of reagent 3.
5) Anhydrous Sodium Sulfate.
6) 0.1 N Sodium Hydroxide.
7) 0.01 N Sulfuric Acid.
8) Hydrocortisone : standard solutions containing 10 and 20 μg per milliliter are prepared by dissolving hydrocortisone.

Procedure :

A 24-hour urine specimen was collected and stored in the refrigerator until extraction. A urine specimen of 10 ml was adjusted to pH 1.0 by the dropwise addition of 50% sulfuric acid. Two successive extractions were carried out with the use of 0.5 volume of n-butanol by shaking for five minutes. The n-butanol layer was separated after centrifugation for five minutes at approximately 2500 r. p. m.. The combined extracts were washed twice with 0.2 volume of 0.1 N sodium hydroxide followed by 0.4 volume of 0.01 N sulfuric acid. Each wash was shaken one minute only. The butanol extract was filtrated after dehydration by 0.5 g of anhydrous sodium sulfate. Then colorimetry was performed immediately after completion of the extraction.

Colorimetry. The following tubes were prepared :

A Tubes	B Tubes
4 ml of phenylhydrazine-sulfuric acid reagent was added to :	4 ml of 20.5 N sulfuric acid reagent was added to :
1 ml butanol extract (sample A)	1 ml butanol extract (sample B)
1 ml butanol (blank A)	1 ml butanol (blank B)
1 ml hydrocortisone solution (standard A)	1 ml hydrocortisone solution (standard B)

The tubes were thoroughly mixed and incubated in a constant temperature water bath at 60°C for exactly 30 minutes after which they were transferred to a cold water bath for five minutes. The optical density was read at 410 millimicrones on a spectrophotometer (*SHIMAZU* QB-50) set at zero with a distilled water blank.

Calculations :

Sample A − Sample B = (a)
Blank A − Blank B = (b)

(a) − (b) corrected optical density of the unknown sample. The corrected optical density of the standard was derived in the same manner.

Then :

$$17\text{-OHCS (mg/24 hrs.)} = \frac{\mu g/ml \text{ standard}}{\text{C. O. D. standard}} \times \text{C. O. D. sample} \times 24 \text{ hrs. urine volume.}$$

(C. O. D. = corrected optical density)

ii) Method for Determination of Urinary Free 17-OHCS

Twenty ml of urine was extracted twice at pH 1.0 with 10 ml of chloroform. The combined chloroform extracts were washed successively with 4 ml each of 0.1 N NaOH and 0.01 N H_2SO_4. The extract was dried over 2.0 mg of anhydrous sodium sulfate and filtered through glass wool. The filtrate was evaporated to dryness at room temperature under a stream of air. The residue was dissolved in 3 ml of n-butanol and the colorimetric preocedure was carried out as described above. The calculation was altered only by the concentration factor achieved by evaporation.

iii) Method for Determination of Serum Polyenoic Acids

The polyenoic acids in serum were determined by the alkaline isomerization method of HOLMAN and HAYES, improved by our colleague JINDO.

3. EXPERIMENTAL OBJECTS

The examination was performed in 11 cases of healthy adults (control group), in 10 patients with gastric ulcer, in 10 patients with gastric cancer, in 6 patients with esophageal cancer, in 14 patients with gallstones, and in other 8 patients.

It is known that the liver is an important organ which takes part in the metabloism of corticoids, and the disturbance of liver function impairs mainly the glucuronic acid conjugation of plasma free 17-OHCS and then affects the excretions of urinary 17-OHCS. Accordingly, surgical patients without a distinct disturbance of liver function were taken for this study.

4. RESULTS

The results from this examination are shown in Table 9.

The evaluation of adrenocortical capacity by ACTH test was estimated from the increment of urinary 17-OHCS excretions. The excretions of free 17-OHCS increased in proportion to the changes of total values. The values of free form were generally about from 5 to 10 per cent of the total amount of 17-OHCS.

The gas-liquid chromatographic investigations in our laboratory clarified that in the human serum, the dienoic acid represents linoleic acid, and the tetraenoic acid represents mostly arachidonic acid and docosatetraenoic acid. Since dienoic acid and tetraenoic acid have the peculiar physiological significances as EFA, also in the present experiments, the relationship of these acids to the adrenocortical capacity of various surgical patients was investigated.

i) Relationship between Serum EFA Levels and Adrenocortical Capacity in Surgical Patients

In the various surgical patients except those with gallstones, the correlation of their serum EFA levels to the increment of total 17-OHCS excretions, induced by ACTH injection, is shown in Fig. 7.

The parallel relation between an amount of serum EFA and adrenocortical capacity of these patients was observed also in the clinical cases, and it was recognized that the adrenocortical capacity of the individuals, whose EFA levels in serum were decreased, was extremely reduced.

Table 9

No.	Name	Age	Sex	Diseases	Serum Polyenoic Acids (mg/dl)					Urinary 17-OHCS mg/24 hrs.					
										Before ACTH		After ACTH		Increment	
					Di	Tr	Tt	Pt	Hx	Free	Total	Free	Total	Free	Total
1	T.G.	30	M	Healthy	61.6	8.3	13.2	2.5	4.7	0.12	3.1	0.84	19.9	0.72	16.1
2	Y.F.	30	M	〃	48.5	3.2	8.5	1.8	2.6	0.03	6.5	0.95	16.8	0.92	10.3
3	O.K.	45	M	〃	67.0	12.5	14.0	1.2	3.9	0.07	4.4	0.54	19.0	0.47	14.6
4	K.F	38	M	〃	58.8	8.0	9.9	3.8	6.8	0.14	1.5	1.27	14.5	1.13	13.0
5	U.F.	31	M	〃	52.0	6.3	11.2	1.9	4.8	0.26	5.5	1.22	21.0	0.96	15.5
6	S.T.	45	F	〃	45.5	6.7	9.4	2.4	4.3	0.01	1.8	1.98	16.9	1.97	15.1
7	H.H.	55	M	〃	42.5	7.8	13.5	1.1	2.9	0.09	5.1	0.76	18.1	0.67	13.0
8	Y.T.	51	M	〃	59.8	7.5	12.0	1.6	3.4	0.15	3.9	1.43	25.9	1.28	22.0
9	R.S.	49	F	〃	61.2	4.5	8.5	1.3	4.9	0.09	2.8	1.12	18.8	1.03	16.0
10	O.S.	30	F	〃	51.3	4.7	7.5	2.8	5.5	0.12	2.3	1.25	12.8	1.13	10.5
11	T.U.	20	F	〃	47.2	4.4	8.0	0.5	3.8	0.13	4.9	1.44	15.0	1.31	10.1
12	G.K.	40	M	Gastric Ulcer	60.1	4.2	6.8	3.3	5.1	0.50	5.5	0.96	28.0	0.46	22.5
13	K.N.	38	F	〃	36.2	8.5	6.8	2.8	4.6	0.08	4.1	0.42	12.1	0.34	8.0
14	F.A.	50	F	〃	40.0	7.9	9.7	3.8	4.5	0.21	3.2	1.01	15.2	0.80	12.0
15	U.Y.	52	M	〃	58.0	4.0	7.3	3.2	6.1	0.08	4.7	0.99	14.5	0.91	9.8
16	H.K.	25	M	〃	31.3	7.0	8.2	2.3	5.9	0.19	4.4	1.21	15.0	1.02	10.6
17	O.Y.	40	M	〃	45.8	3.9	8.2	1.2	2.8	0.03	3.3	1.31	13.5	1.28	10.2
18	A.N.	59	M	〃	57.0	1.7	15.5	1.7	3.3	0.92	3.0	1.33	17.7	0.41	14.7
19	E.S.	64	M	〃	47.0	2.9	9.3	3.1	4.8	0.11	2.0	1.08	13.5	0.97	11.5
20	K.Y.	53	F	〃	45.0	3.8	10.3	1.9	2.8	0.03	2.1	0.76	14.6	0.73	12.5
21	T.T.	48	F	〃	43.5	4.2	10.7	2.3	4.9	0.52	2.5	0.95	15.5	0.43	13.0
22	O.Y.	48	M	Gastric Cancer	39.0	4.8	5.0	2.5	4.7	0	2.1	0.42	8.3	0.42	6.2
23	S.K.	55	M	〃	37.5	4.7	7.6	2.8	3.5	0.12	2.3	0.82	15.8	0.70	13.5
24	H.K.	45	M	〃	35.0	4.1	5.5	1.3	3.8	0.19	3.8	0.22	6.8	0.03	3.0
25	K.Y.	64	M	〃	29.0	4.2	6.9	1.9	2.8	0.2	4.2	0.38	9.5	0.18	5.3
26	S.T.	58	M	〃	36.5	6.6	11.0	1.8	4.2	0.19	3.7	0.97	22.8	0.78	19.1
27	I.T.	57	M	〃	20.6	3.0	4.3	2.0	3.5	0.12	2.9	0.32	6.5	0.20	3.6
28	D.A.	63	F	〃	38.0	5.5	4.8	1.1	2.1	0.09	3.1	0.41	11.4	0.32	8.3
29	H.M.	45	M	〃	36.0	4.1	7.7	2.4	5.6	0.01	8.6	0.81	16.6	0.80	8.0
30	R.N.	37	F	〃	24.2	2.5	8.0	1.2	2.5	0.12	3.0	1.0	13.3	0.88	10.3
31	U.T.	35	F	〃	27.5	5.5	6.4	2.7	3.8	0.08	1.5	0.99	8.6	0.91	7.1
32	T.U.	59	M	Esoph. Cancer	33.2	2.8	5.0	1.3	3.8	0.10	3.2	0.13	8.2	0.03	5.0
33	I.M.	60	F	〃	60.0	5.1	12.6	3.1	3.0	0.20	6.1	0.94	28.8	0.74	22.7
34	S.T.	64	M	〃	54.5	3.6	13.5	2.6	6.1	0.05	3.3	0.71	16.5	0.66	13.2
35	G.I.	75	M	〃	24.0	2.9	5.6	1.8	3.9	0.13	2.4	0.88	10.0	1.35	7.6
36	Y.K.	52	M	〃	32.5	3.1	6.2	3.3	3.8	0.17	3.4	0.87	8.1	1.10	4.7
37	R.O.	65	M	〃	46.0	4.2	9.5	2.9	4.1	0.08	3.0	1.18	15.5	1.10	12.5
38	O.T.	50	M	Gallstones	48.0	4.5	7.2	1.8	2.6	0.21	4.7	0.35	11.0	0.14	6.3
39	K.K.	57	F	〃	50.0	10.3	9.8	2.5	4.7	0.52	0.1	0.76	5.1	0.24	5.0
40	I.G.	36	F	〃	48.0	4.3	7.9	1.4	3.9	0.29	6.5	0.81	14.5	0.52	8.0

41	T.K.	40	F	〃	49.0	3.9	9.4	1.2	2.5	0.12	1.5	0.99	6.0	0.87	4.5
42	M.F.	62	F	〃	47.6	1.9	5.9	5.2	6.8	0.19	2.7	0.48	6.9	0.29	1.2
43	U.F.	60	M	〃	37.1	6.9	8.8	3.8	3.0	0.09	3.2	0.41	8.5	0.32	5.3
44	Y.K.	43	F	〃	40.0	3.4	7.4	2.4	1.5	0.25	5.0	0.90	18.2	0.65	13.2
45	K.K.	64	F	〃	41.1	4.7	6.6	1.6	3.4	0.92	2.1	1.02	7.7	0.10	5.6
46	K.M.	41	F	〃	39.0	3.7	6.1	1.3	3.8	0.09	3.3	1.12	9.0	1.03	5.7
47	E.H.	68	F	〃	43.7	6.2	8.7	1.2	7.6	0.13	3.7	0.76	15.0	0.63	11.3
48	O.K.	50	F	〃	47.5	5.9	9.0	2.8	2.6	0.26	3.0	1.22	8.8	0.96	5.8
49	H.H.	32	M	〃	49.5	1.2	8.5	3.0	2.1	0.21	3.7	1.01	15.2	0.80	11.5
50	M.A.	48	M	〃	51.7	6.0	8.6	2.0	3.9	0.20	3.0	0.80	9.5	0.60	6.5
51	N.N.	52	F	〃	38.9	4.4	7.2	2.0	3.5	0.34	3.6	0.84	11.5	0.50	7.6
52	T.I.	45	M	Gastritis	17.8	2.6	7.7	1.7	3.8	0.33	3.5	1.25	20.7	0.92	17.2
53	S.K.	25	M	Enterostomy	32.0	2.9	6.0	2.6	1.1	0.22	2.9	0.95	8.2	0.73	5.3
54	G.Y.	35	F	Ileus	29.4	1.1	10.8	3.1	4.5	0.04	2.0	1.06	15.8	1.02	13.8
55	H.R.	25	M	Elephantiasis	32.0	5.2	4.5	1.2	3.8	0.27	2.5	1.98	8.0	1.71	5.5
56	R.N.	69	M	Hepatic Cancer	39.8	4.3	5.9	3.5	4.0	0.15	3.6	0.76	14.0	0.61	10.4
57	M.Y.	36	F	Cholecystitis	49.0	5.8	8.6	1.9	4.3	0.22	5.7	2.01	15.8	1.76	10.1
58	H.K.	55	M	〃	29.0	3.1	4.2	1.4	1.4	0.19	2.5	1.43	7.9	0.24	5.4
59	Y.K.	40	F	〃	57.1	5.5	7.4	4.9	3.0	0.15	4.5	1.27	18.0	1.12	13.5

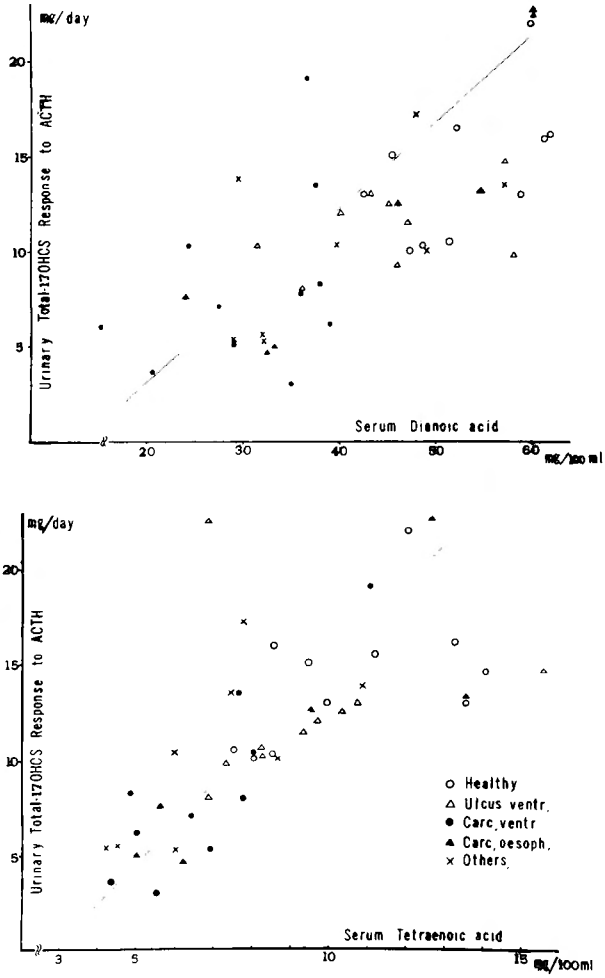

Fig. 7 Relationship between Serum EFA Levels and Adrenocortical Capacity in Surgical Patients.

ii) Relationship between Serum EFA Deficient Groups and Their Adrenocortical Capacity

The serum EFA levels above or below the lower limits of the two standard deviations from the mean in healthy adults were evaluated as the sufficient group or the deficient group. That is, serum dienoic acid levels above or below 40.0 mg per 100 milliliter were proposed as sufficient or deficient, and serum tetraenoic acid levels above or below 6.0 mg per 100 milliliter, as sufficient or deficient.

Then the urinary 17-OHCS response to ACTH injection of both groups was compared. Little difference was observed between EFA sufficient and deficient groups in urinary 17-OHCS excretions at rest, however, the increment of urinary 17-OHCS excretions, induced by ACTH injection, was significantly greater in the EFA sufficient group than in the EFA deficient group, and on the tetraenoic acid, especially, a marked difference was observed between these two groups (Fig. 8).

Then, from the same proposed criteria as described above, the increment of 17-OHCS excretions, induced by ACTH injection, above or below 7.0 mg per day was evaluated as normal or reduced adrenocortical capacity (Table 10).

Group I: The state at which the amounts of dienoic acid and tetraenoic acid in serum are still sufficient, and the adrenocortical capacity is completely normally maintained.

Group II: The slightly deficient state in the total amount of EFA, but since tetraenoic acid, which is derived from dienoic acid, is sufficiently present the adrenocortical capacity is generally normally maintained.

Group III: The exhaustive state in which the amout of dienoic acid as well as of

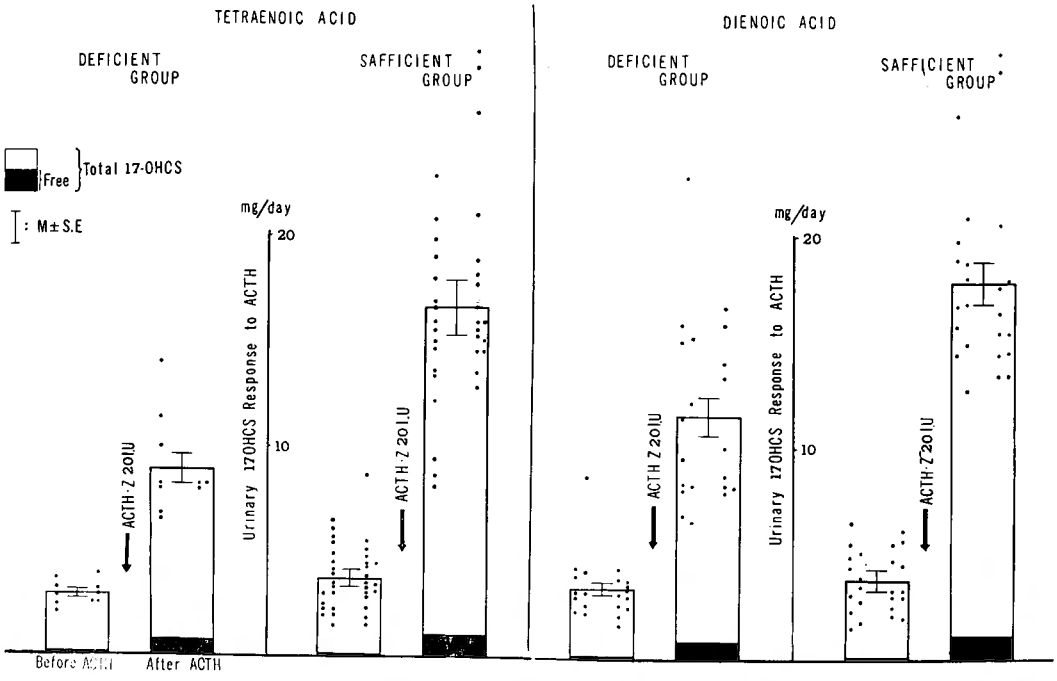

Fig. 8 Relationship between Serum EFA Deficient Groups and Their Adrenocortical Capacity

Table 10 Relationship between Serum EFA Deficiency and Reduced Adrenocortical Capacity

Group	Serum EFA	Cases	Cases of Reduced Adrenocortical Capacity (%)
I	Di-sufficient Tt-sufficient	24	0 (0)
II	Di-deficient Tt-sufficient	11	2 (18.1)
III	Di-deficient Tt-deficient	10	7 (70)
IV	Di-sufficient Tt-deficient	0	0 (0)

tetraenoic acid in the serum is decreased, and the adrenocortical capacity is reduced for the most part.

Group IV : Completely absent state, of the cases observed none fell under this group.

That is, when individuals are exposed to various kinds of stresses, the mobilization of the *depót fat*, in which dienoic acid has been previously stored, occurs. And then, the decreased adrenal EFA contents, caused by stresses, is complemented from the mobilized dienoic acid. Accordingly, it is said that the adrenocortical capacity is almost normally maintained because tetraenoic acid is sufficiently synthesized from this dienoic acid.

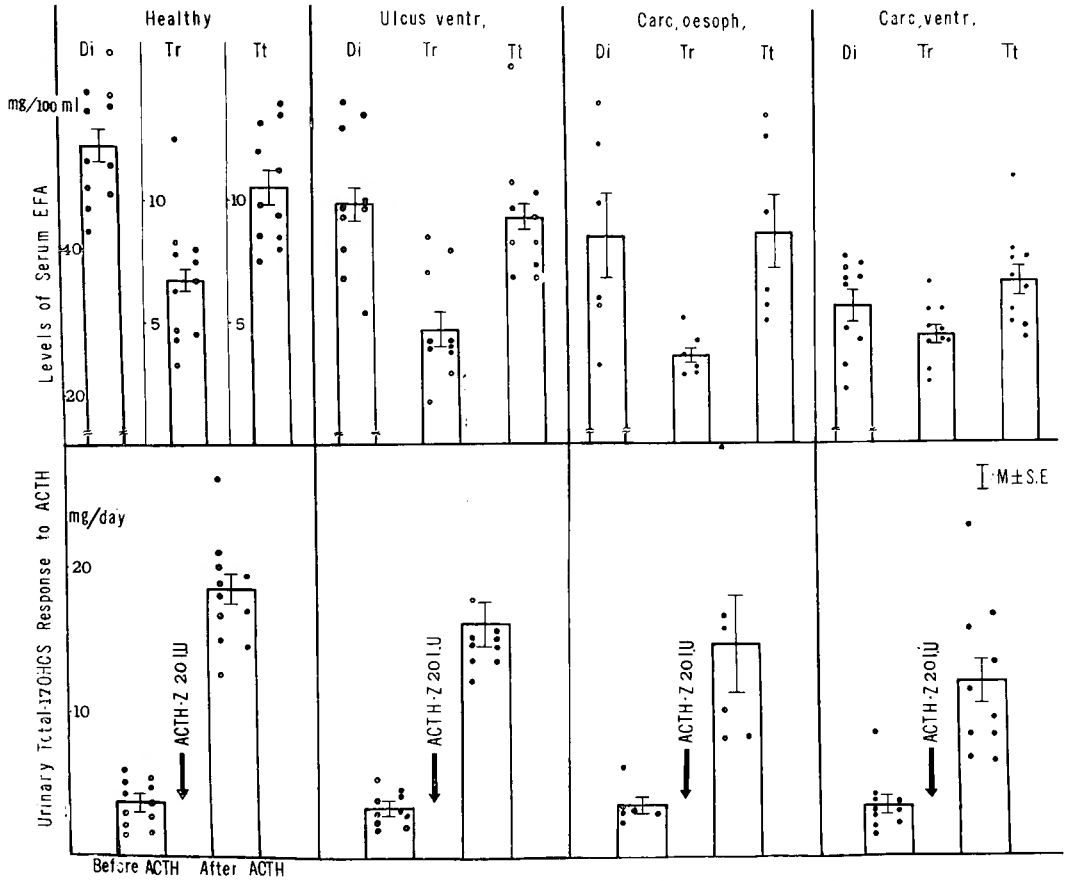

Fig. 9 Relationship between Serum EFA and Adrenocortical Capacity in Various Surgical Diseases

iii) Relationship between Serum EFA Levels and Adrenocortical Capacity in the Various Surgical Diseases

In gastric ulcer, esophageal cancer and gastric cancer groups, the serum dienoic acid and tetraenoic acid were decreased in quantity as compared with those of healthy group (Fig. 9).

Also parallel with the decrease in EFA, the suppression in the adrenocortical capacity was observed. That is, in these observations, an amount of EFA in the serum of these surgical patients showed a parallel relationship to their adrenocortical capacity.

In the patients with cancerous diseases, especially gastric cancer cases, the marked decrease in serum EFA levels was observed. And it is thought that such changes in EFA might be caused from the increased consumption of EFA in the body due to the rapid growth of the malignant tumor, and partly from the decreased supply of dietary EFA.

Therefore the reduced adrenocortical capacity in the cancerous patients may be caused from the disturbed biosynthesis of adrenal steroid hormone due to the disturbances in adrenal cholesterol metabolism by the decreased amount of EFA in the body.

iv) Relationship between Serum EFA Levels and Adrenocortical Capacity in the Patients with Gallstones

In comparison with the serum EFA levels of healthy adults, the serum EFA levels, especially tetraenoic acid levels, of 14 patients with gallstones without distinct disturbance of liver function, were clearly decreased. And their adrenocortical capacity was also greatly suppressed, regardless of the kind of stone, and the grade of suppression was greater than that of the gastric cancer cases (Fig. 10). These facts suggest that cholelithiasis is a disease induced under peculiar metabolic disturbances in EFA.

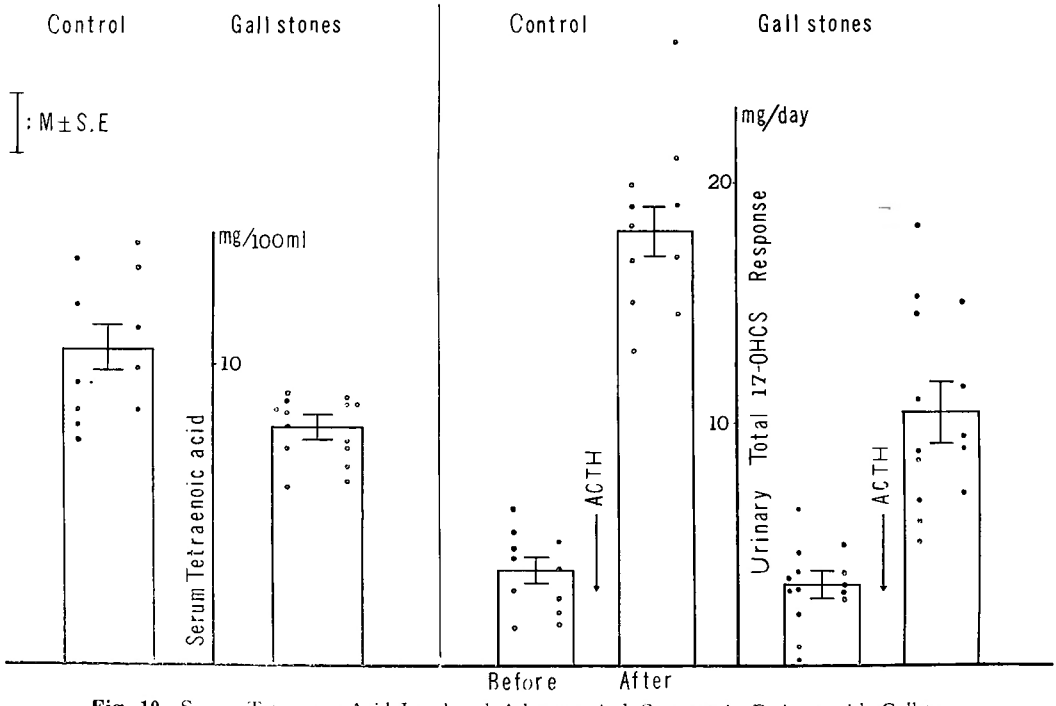

Fig. 10 Serum Tetraenoic Acid Level and Adrenocortical Capacity in Patients with Gallstones

5. DISCUSSION

Up to the present, in our laboratory, the pathologic physiology on the fat metabolism in the surgical field has been investigated, using the alkaline isomerization method and gas-liquid chromatography for the chemical analysis.

In the present study, the correlation of serum tetraenoic acid levels in surgical patients to their adrenocortical capacity was studied. And it was clarified that in human beings, the degree of adrenocortical capacity was finally prescribed from an amount of tetraenoic acid with which the adrenal cholesterol esterifies.

But it was demonstrated by co-worker MURAOKA that in the human adrenals, docosatetraenoic acid and also arachidonic acid was present in larger quantities than in the rat adrenals. Accordingly, in human beings, it is impossible to assert that the degree of adrenocortical capacity is prescribed from an amount of cholesteryl arachidonate in the adrenals as in the rats. And it is appropriate to consider that the adrenocortical capacity in human beings is prescribed from an amout of tetraenoic acid with which adrenal cholesterol esterifies. However, since docosatetraenoic acid is synthesized from arachidonic acid in the body the physiological significances of these two acids may be considered almost identical.

Moreover, in the emaciated patients with cancer, marked deficiency in EFA was found, and naturally in such patients, reduced biosynthesis of adrenal steroid hormone was observed through the disturbed activation of adrenal cholesterol.

On the other hand, nowadays, the initiating factors of gallstones are not yet completely known, but the fact that decreased levels in serum tetraenoic acid and reduced adrenocortical capacity were always observed in the patients with gallstones, suggests that this disease may be caused by the peculiar metabolic disturbances in EFA.

6. CONCLUSION

i) The parallel relationship between the serum EFA levels, especially tetraenoic acid levels, and adrenocortical capacity in the surgical patients was observed.

ii) The decreased levels in serum EFA attributed to the existence of a malignant tumor were found in the emaciated patients with cancer.

iii) In the patients with gallstones, the decreased tetraenoic acid levels in serum and the reduced adrenocortical capacity were observed, and these facts suggest that cholelithiasis may be caused by the peculiar metabolic disturbances in EFA.

iv) In human beings also, it was clarified that the individual adrenocortical capacity was prescribed by the quantity of tetraenoic acid contained in the body.

v) In the human adrenals, docosatetraenoic acid and also arachidonic acid were abundantly present, but since the former is derived from the latter in the body, the physiological significances of these two acids may be considered almost identical.

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和文抄録

副腎皮質予備能力に於ける不可欠脂酸欠乏の意義についての実験的並びに臨床的研究

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従来我々は，外科的立場から，不可欠脂酸（EFA）の特殊生理学的意義の解明に努めて来た．そして，我々は既に生体内で単位容積当り最も多量の EFA を含有する臓器が副腎であり，而も同時に副腎中にはcholesterol も亦多量に含有されているところから，EFA が必ずや副腎の cholesterol 代謝に与り，その終末代謝産物である steroid hormone の生合成にそれが何等かの重要な役割を果たしているものと考えた．而してこのような考え方が果たして妥当なものであるかどうかについて従来光学並びに電顕学的研究方法あるいは生化学的研究方法により検討を加えて来たが，更に本研究に於ても，まず基礎的に，EFA 及び Vit. B_6 の欠乏状態が副腎の glucocorticoids の産生に如何なる程度の影響を及ぼすものであるかを，ラッテを用いて検討すると共に，共同研究者村岡によつて測定され得た副腎中の cholesterol 及び EFA 量の推移とも併せ考え，次のような結論に到達した．即ち，

1） 安静時既に，EFA の欠乏した個体に於ては血清及び副腎中の corticosterone 濃度は明らかに低下している．そして，副腎中の total 及び ester 型 cholesterol の減少はみられないが，colesteerol と ester 結合している arachidonic acid は明らかに減少していた．

2） ACTH 注射及び寒冷刺戟により，EFA の欠乏した個体に於ては，その adrenocortical capacity が著しく低下していることを立証し得た．一般に stress が作用すると，副腎中の total 及び ch-ester arachidonic acid は何れも減少するものではあるが，EFA の欠乏した個体では特にその程度が著しく，結局は exhaustive な状態へと移行したのに対して，EFA を充分保有している個体では，一旦 total 及び ch-ester arachidonic acid が減少してもそれらは直ちに速かに旧に復する傾向を示した．

3） ACTH-Z を連続4回に亘り注射した際の血清及び副腎中の corticosterone の示す態度を検討すると，Vit. B_6 欠乏 EFA 投与群は，第1回目注射時には，Vit. B_6 投与 EFA 投与群と略同様に充分な反応力を示すが，その後 ACTH-Z 注射回数の増加と共に漸次その反応力は減弱し，第4回目の注射に際しては，最早 Vit. B_6 投与 EFA 欠乏群と略同様の反応力を示すに過ぎなかつた．そして，亦副腎中の arachidonic acid 量の推移をみると，第1回目注射時には，Vit. B_6 投与 EFA 投与群と同様に未だ充分量の total 及び ch-ester arachidonic acid を保有しているが，第4回目の注射時には，total arachidonic acid のみはなお充分に保有されているが，ch-ester arachidonic acid は選択的に明らかな減少を来たしていた．

4） 従つて，adrenocortical capacity の良否は，副腎中に含有される total 及び ester 型 cholesterol の量に左右されるものではなくして，cholesterol と ester 結合して存在する arachidonic acid 量の如何によつて規定され得るものと考えて然るべきである．そこで，更に以上のような基礎的研究によつて得られた事実が，果たして人体にも適用し得るものであるかどうかを臨床的立場から検討する目的で，京都大学医学部外科第2講座に入院中の外科的患者の中から適当な症例を選び，それらの血清中のEFA濃度と，adrenocortical capacity との相関性を，ACTH-test によつて検討してみた．その結果，

1） 臨床例に於ても明らかにその血清中の EFA 濃度，特に tetraenoic acid 濃度と，その adrenocortical capacity とはよく平行的関係を示し，血清中の tetraenoic acid 濃度が減少した個体に於ては，その adrenocortical capacity も亦著明に低下していた．

2） 癌患者に於ては，一般に腫瘍の存在によつて，その血清中のEFA濃度は減少し，同時にその adrenocortical capacity も必然的に低下していた．

3） 胆石症に於ては，結石の種類の如何に拘らず，全てその adrenocortical capacity は低下して居り，同時にその血清中の tetraenoic acid 濃度も亦減少していた．このような事実のみからすれば，胆石症なるものは EFA の特異な代謝異常下に於て発生する一疾患である事が推測される．